

Whole Genome Sequencing of *Legionella* species

Utah Legionnaires' Disease Prevention Conference

Dr. Kelly F. Oakeson

June 27, 2019

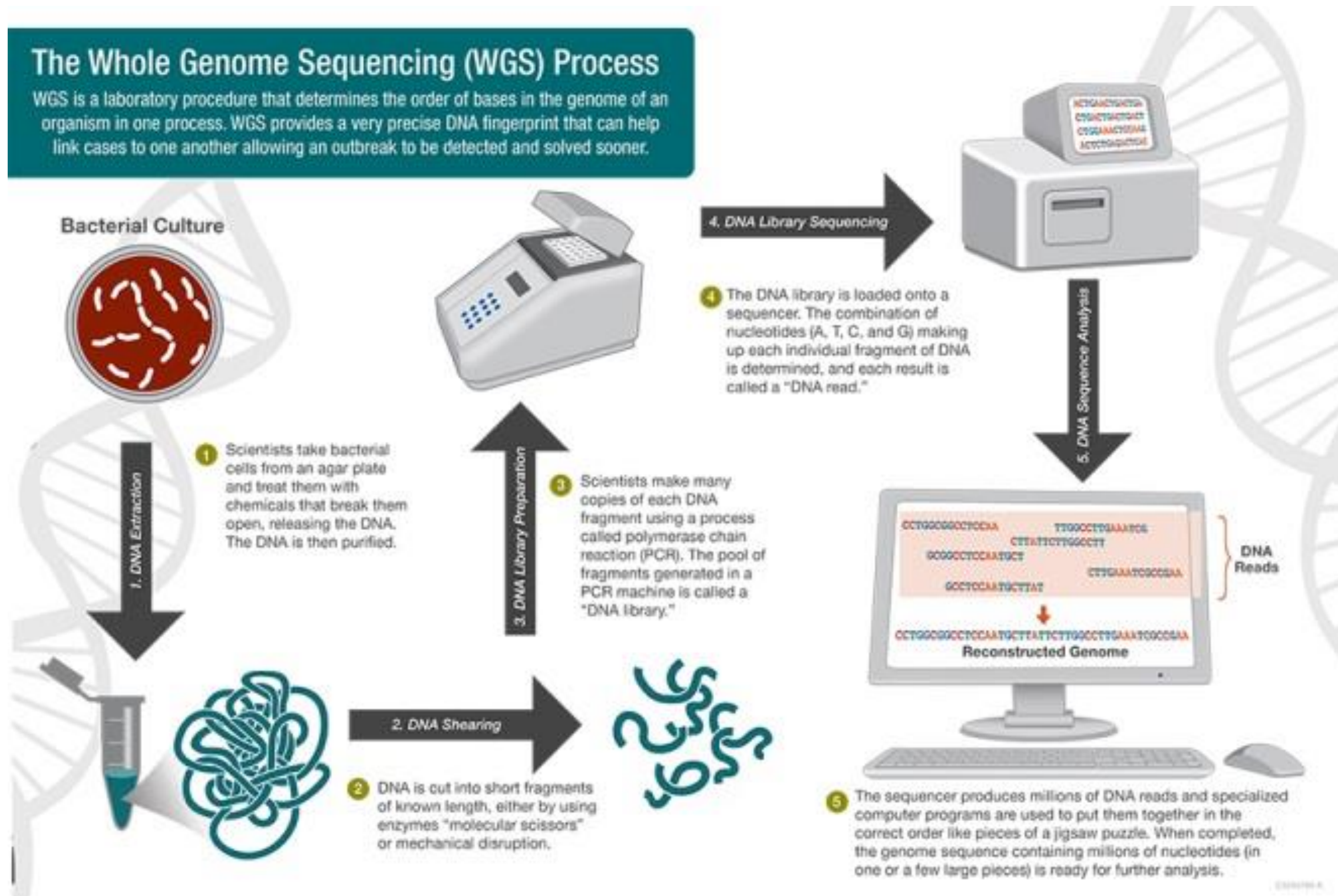


UTAH DEPARTMENT OF
HEALTH



Utah Public Health
Laboratory

WGS Process



WGS for *Legionella*



Applied and Environmental
Microbiology



Genomic Resolution of Outbreak-Associated *Legionella pneumophila* Serogroup 1 Isolates from New York State

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ABSTRACT

A total of 30 *Legionella pneumophila* serogroup 1 isolates representing 10 separate legionellosis laboratory investigations (“outbreaks”) that occurred in New York State between 2004 and 2012 were selected for evaluation of whole-genome sequencing (WGS) approaches for molecular subtyping of this organism. Clinical and environmental isolates were available for each outbreak and were initially examined by pulsed-field gel electrophoresis (PFGE). Sequence-based typing alleles were extracted from WGS data yielding complete sequence types (ST) for isolates representing 8 out of the 10 outbreaks evaluated in this study. Isolates from separate outbreaks sharing the same ST also contained the fewest differences in core genome single nucleotide polymorphisms (SNPs) and the greatest proportion of identical allele sequences in a whole-genome multilocus sequence typing (wgMLST) scheme. Both core SNP and wgMLST analyses distinguished isolates from separate outbreaks, including those from two outbreaks sharing indistinguishable PFGE profiles. Isolates from a hospital-associated outbreak spanning multiple years shared indistinguishable PFGE profiles but displayed differences in their genome sequences, suggesting the presence of multiple environmental sources. Finally, the *rtx* gene demonstrated differences in the repeat region sequence among ST1 isolates from different outbreaks, suggesting that variation in this gene may be useful for targeted molecular subtyping approaches for *L. pneumophila*. This study demonstrates the utility of various genome sequence analysis approaches for *L. pneumophila* for environmental source attribution studies while furthering the understanding of *Legionella* ecology.

IMPORTANCE

We demonstrate that whole-genome sequencing helps to improve resolution of *Legionella pneumophila* isolated during laboratory investigations of legionellosis compared to traditional subtyping methods. These data can be important in confirming the environmental sources of legionellosis outbreaks. Moreover, we evaluated various methods to analyze genome sequence data to help resolve outbreak-related isolates.

RESEARCH

Legionnaires’ Disease Outbreak Caused by Endemic Strain of *Legionella pneumophila*, New York, New York, USA, 2015

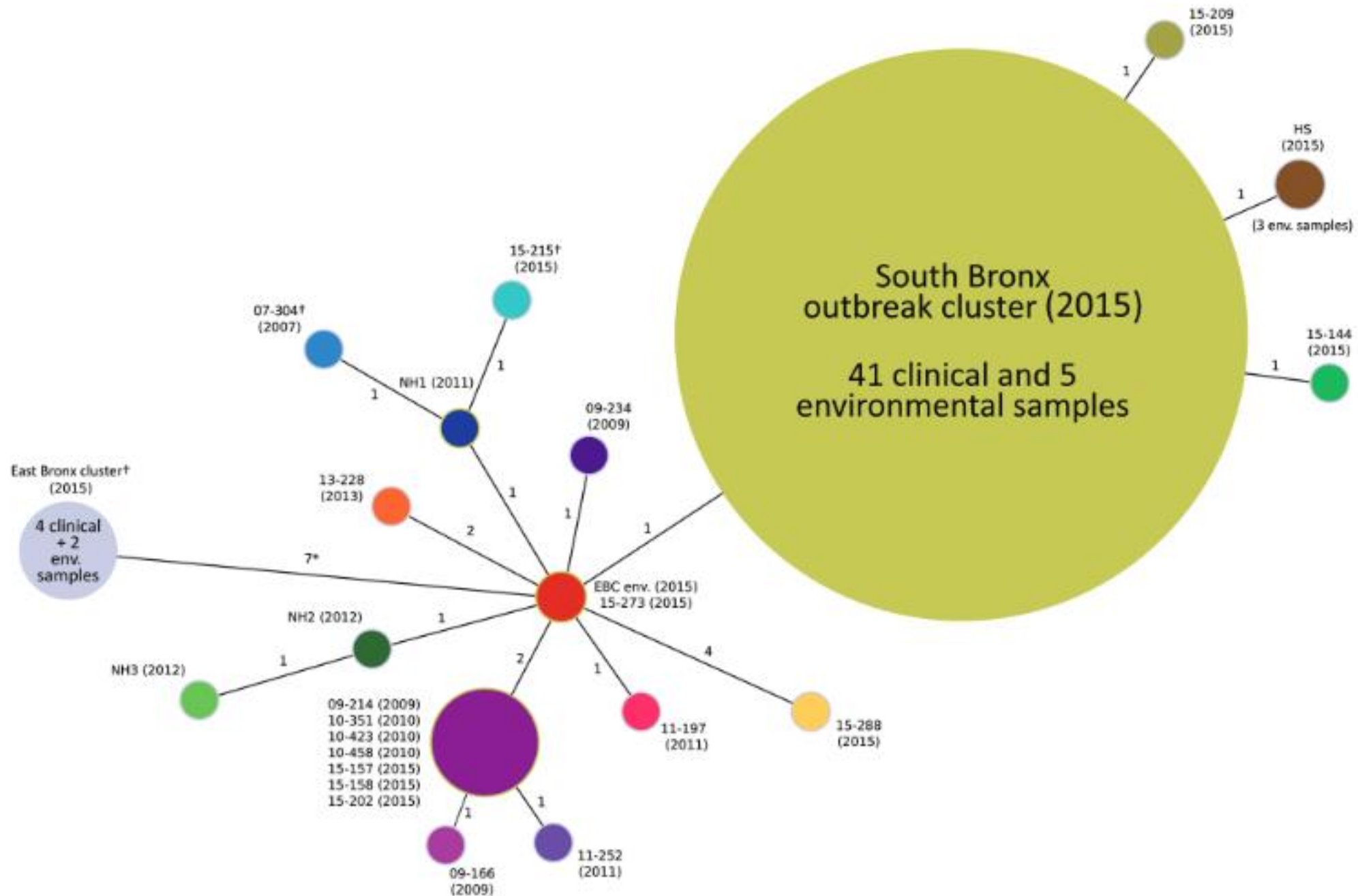
Pascal Lapierre, Elizabeth Nazarian, Yan Zhu, Danielle Wroblewski, Amy Saylor, Teresa Passaretti, Scott Hughes, Anthony Tran, Ying Lin, John Kornblum, Shatavia S. Morrison, Jeffrey W. Mercante, Robert Fitzhenry, Don Weiss, Brian H. Raphael, Jay K. Varma, Howard A. Zucker, Jennifer L. Rakeman, Kimberlee A. Musser

During the summer of 2015, New York, New York, USA, had one of the largest and deadliest outbreaks of Legionnaires’ disease in the history of the United States. A total of 138 cases and 16 deaths were linked to a single cooling tower in the South Bronx. Analysis of environmental samples and clinical isolates showed that sporadic cases of legionellosis before, during, and after the outbreak could be traced to a slowly evolving, single-ancestor strain. Detection of an ostensibly virulent *Legionella* strain endemic to the Bronx community suggests potential risk for future cases of legionellosis in the area. The genetic homogeneity of the *Legionella* population in this area might complicate investigations and interpretations of future outbreaks of Legionnaires’ disease.

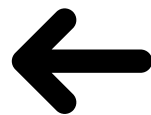
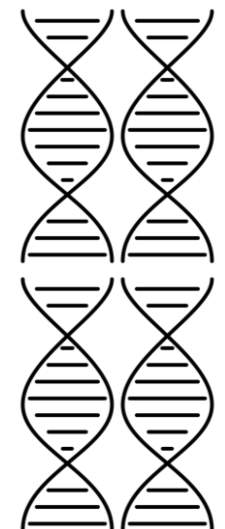
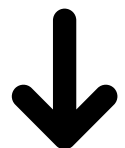
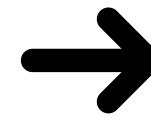
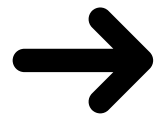
LD was initially detected in 1976, when an outbreak of illness occurred during a meeting of the American Legion in Philadelphia, Pennsylvania, USA; 221 cases were identified, and 34 infected persons died (6). The outbreak, which remains the largest community-associated outbreak of LD in United States, was later linked to the cooling system of the hosting hotel, and a bacterium classified as *L. pneumophila* serogroup 1 was subsequently isolated from 4 persons (7,8).

In the summer of 2015, a large community-associated LD outbreak affected persons who resided or traveled through a large area in the South Bronx region of New York, New York, USA. During July 2–August 2, a total

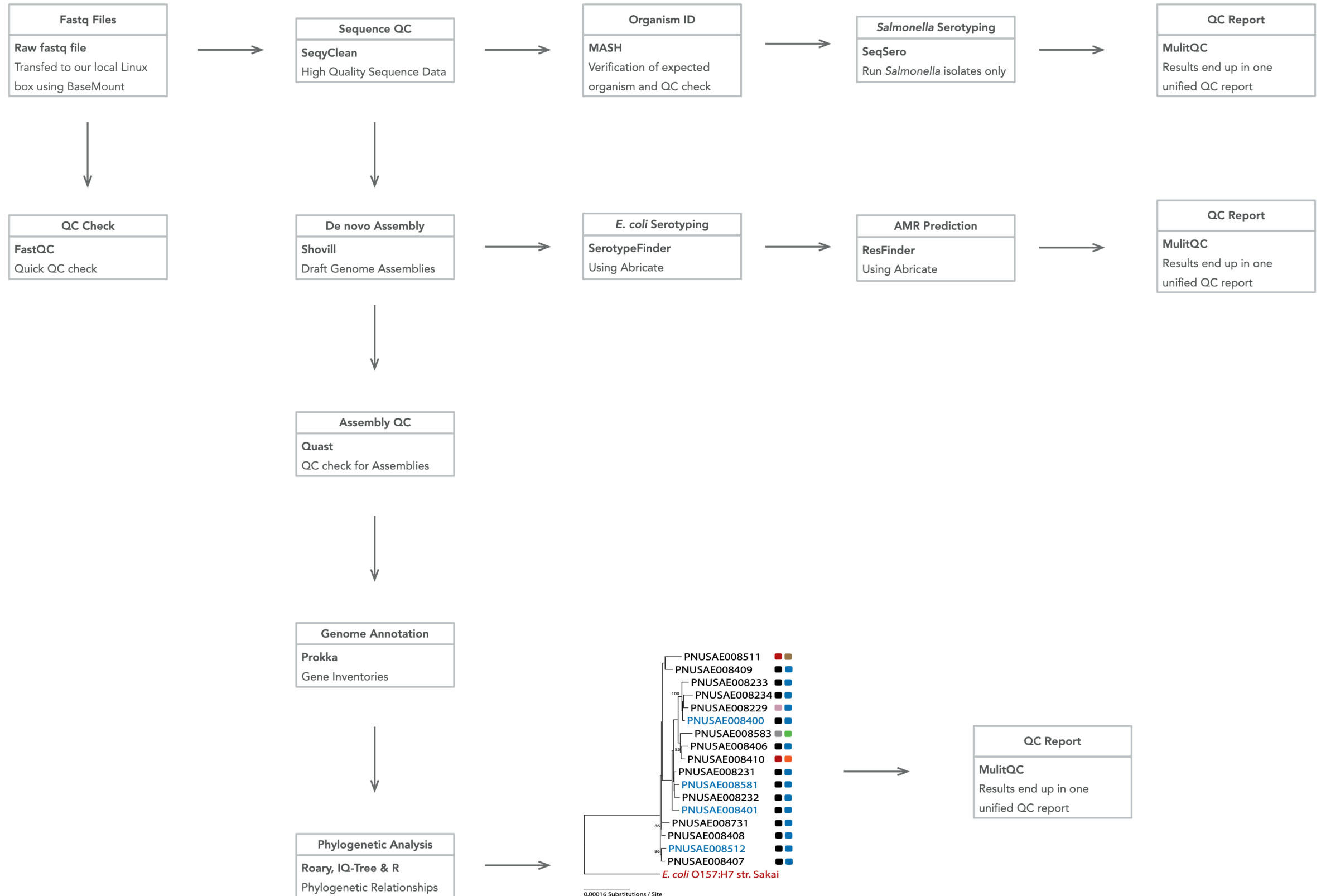
WGS for *Legionella*



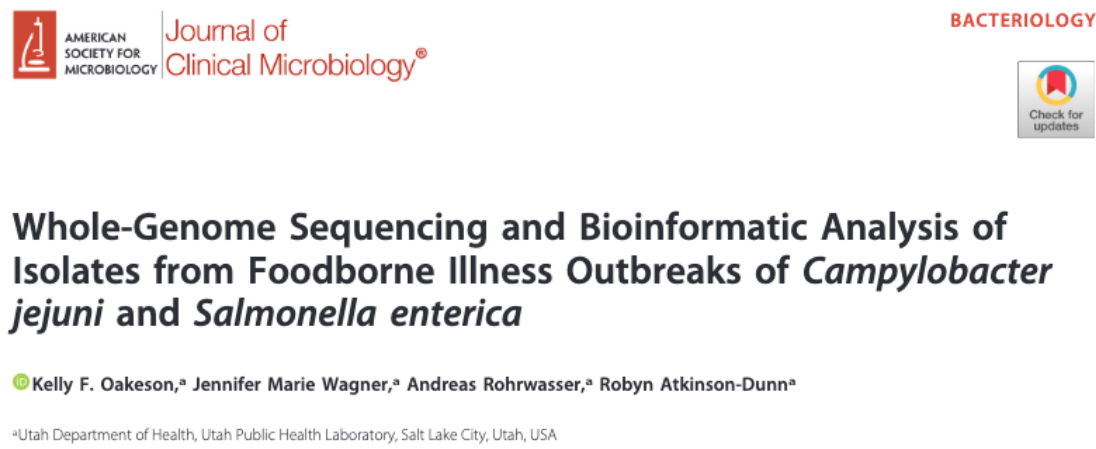
UPHL's Workflow



Reference Free WGS Analysis



Reference Free WGS Analysis



Bioinformatic Analyses of Whole-Genome Sequence Data in a Public Health Laboratory

Kelly F. Oakeson, Jennifer Marie Wagner, Michelle Mendenhall, Andreas Rohrwasser, Robyn Atkinson-Dunn

Morbidity and Mortality Weekly Report

Outbreak of *E. coli* O157:H7 Infections Associated with Exposure to Animal Manure in a Rural Community — Arizona and Utah, June–July 2017

Sarah Luna, PhD¹; Vikram Krishnasamy, MD¹; Louise Saw, MPH^{2,4}; Lori Smith³; Jennifer Wagner, MS³; Jenna Weigand, MPH⁴; Mackenzie Tewell, MA⁵; Marilee Kellis⁵; Roumen Penev, PhD⁵; Laine McCullough, MPH²; Jeffrey Eason, MPH²; Keegan McCaffrey; Cindy Burnett, MPH²; Kelly Oakeson, PhD³; Melissa Dimond, MPH²; Allyn Nakashima, MD²; Deidre Barlow⁶; Anna Scherzer, MS⁶; Melanie Sarino, MPH⁷; Morgan Schroeder, MPH^{8,9}; Rashida Hassan; MSPH^{8,9}; Colin Basler, DVM⁹; Matthew Wise, PhD⁹; Laura Gieraltowski, PhD⁹

Morbidity and Mortality Weekly Report

Sepsis Attributed to Bacterial Contamination of Platelets Associated with a Potential Common Source — Multiple States, 2018

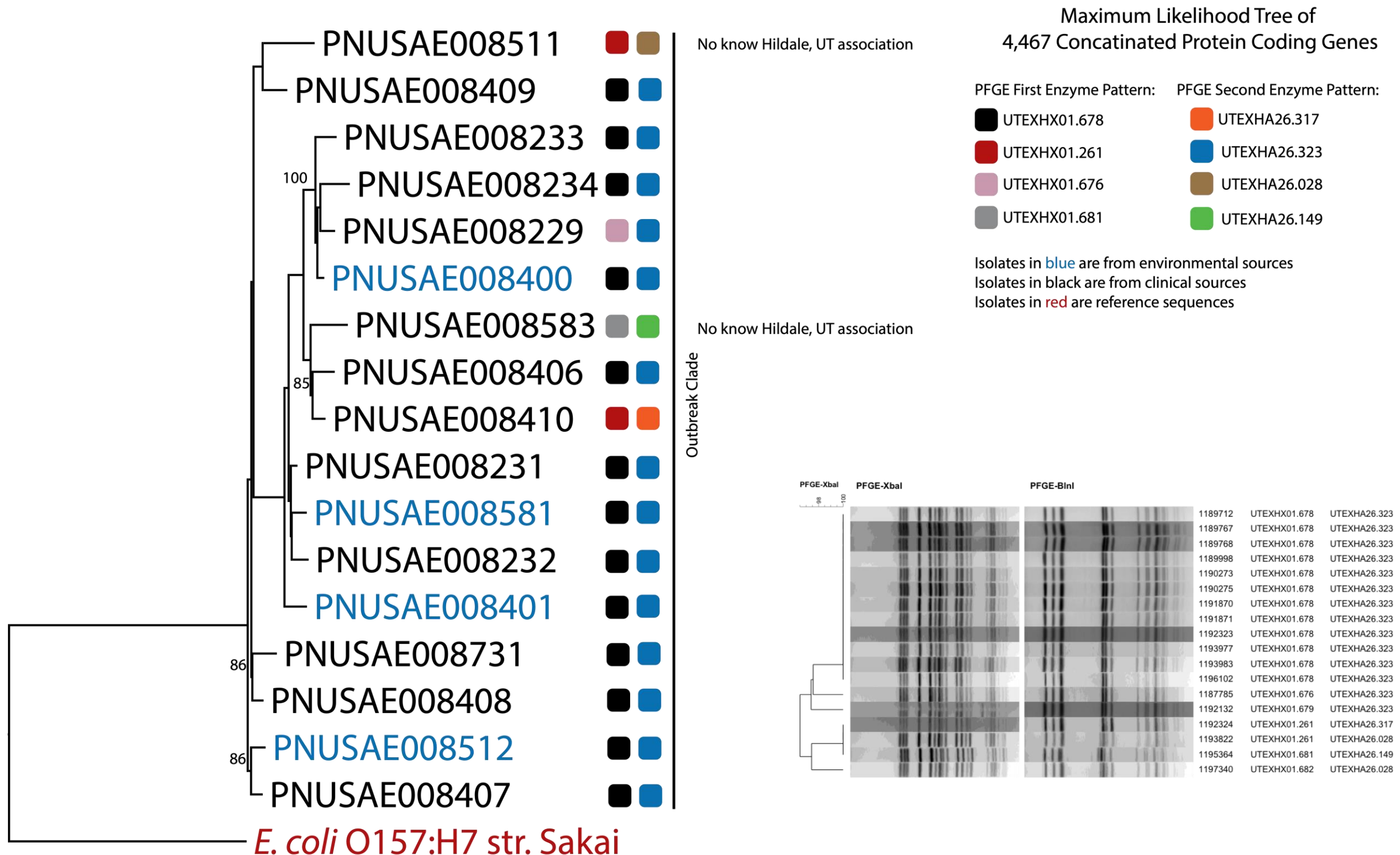
Sydney A. Jones, PhD^{1,2}; Jefferson M. Jones, MD³; Vivian Leung, MD²; Allyn K. Nakashima, MD⁴; Kelly F. Oakeson, PhD⁵; Amanda R. Smith, PhD⁴; Robert Hunter, MS⁶; Janice J. Kim, MD⁶; Melissa Cumming, MS⁷; Eileen McHale⁷; Pampee P. Young, MD, PhD⁸; Joy L. Fridey, MD⁹; Walter E. Kelley, DO¹⁰; Susan L. Stramer, PhD¹¹; Stephen J. Wagner, PhD¹²; F. Bernadette West, MD¹³; Ross Herron, MD⁹; Edward Snyder, MD¹⁴; Jeanne E. Hendrickson, MD¹⁴; David R. Peaper, MD, PhD¹⁴; Adi V. Gundlapalli, MD, PhD^{15,16}; Charles Langelier, MD, PhD^{17,18}; Steve Miller, MD, PhD¹⁷; Ashok Nambiar, MD¹⁷; Morvarid Moayeri, MD, PhD¹⁷; Jack Kamm, PhD¹⁸; Heather Moulton-Meissner, PhD³; Pallavi Annambhotla, DrPH³; Paige Gable³; Gillian A. McAllister³; Erin Breaker, MS^{3,19}; Erisa Sula, MS^{3,19}; Alison Laufer Halpin, PhD³; Sridhar V. Basavaraju, MD³

Morbidity and Mortality Weekly Report

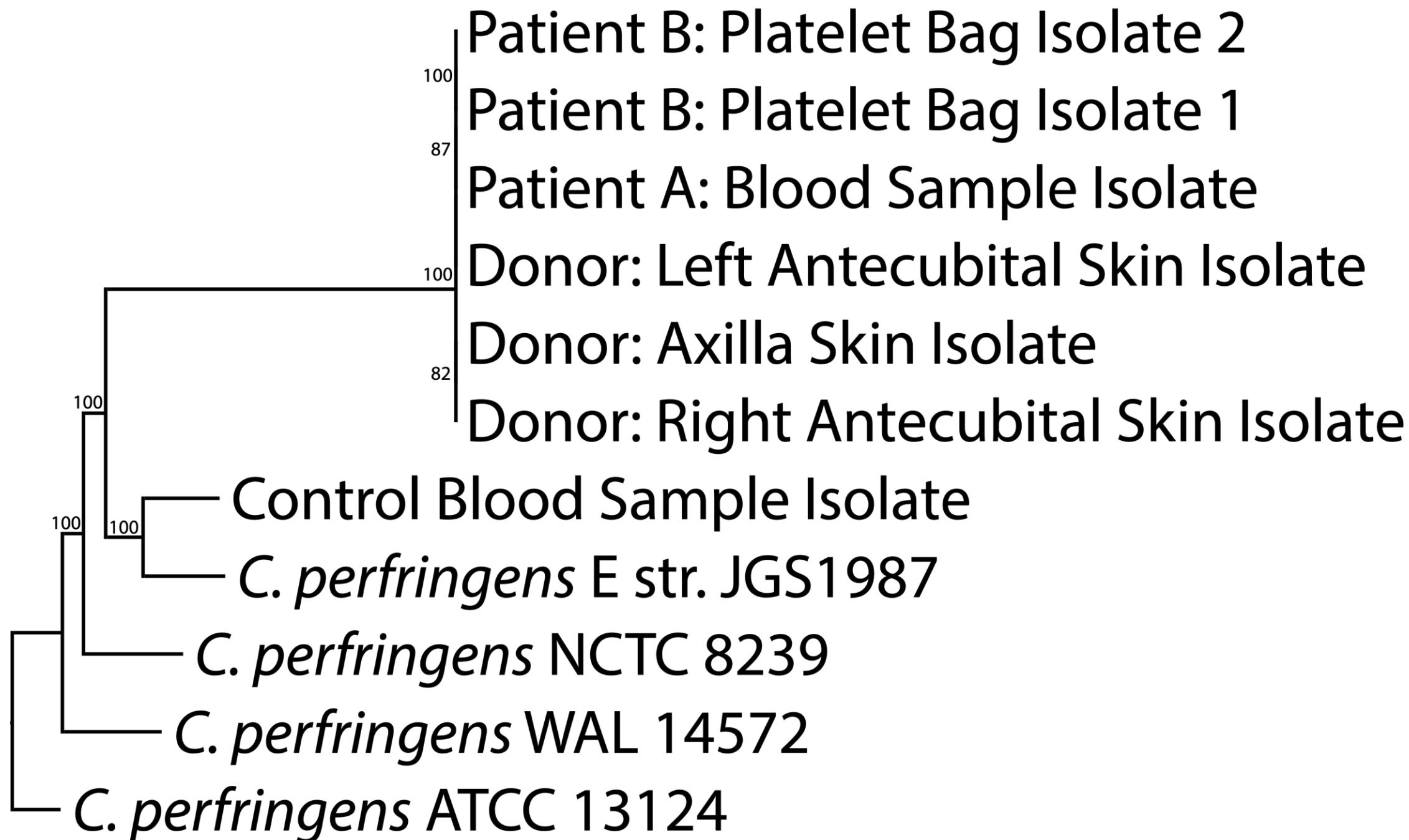
Fatal Sepsis Associated with Bacterial Contamination of Platelets — Utah and California, August 2017

Roberta Z. Horth, PhD^{1,2,3}; Jefferson M. Jones, MD⁴; Janice J. Kim, MD⁵; Bert K. Lopansri, MD⁶; Sarah J. Ilstrup, MD⁶; Joy Fridey, MD⁷; Walter E. Kelley, DO⁸; Susan L. Stramer, PhD⁹; Ashok Nambiar, MD¹⁰; Lynn Ramirez-Avila, MD¹⁰; Amy Nichols, MBA¹⁰; Wendy Garcia¹¹; Kelly F. Oakeson, PhD¹²; Nicholas Vlachos, MS⁴; Gillian McAllister⁴; Robert Hunter, MS⁵; Allyn K. Nakashima, MD³; Sridhar V. Basavaraju, MD⁴

Reference Free WGS Analysis & PFGE

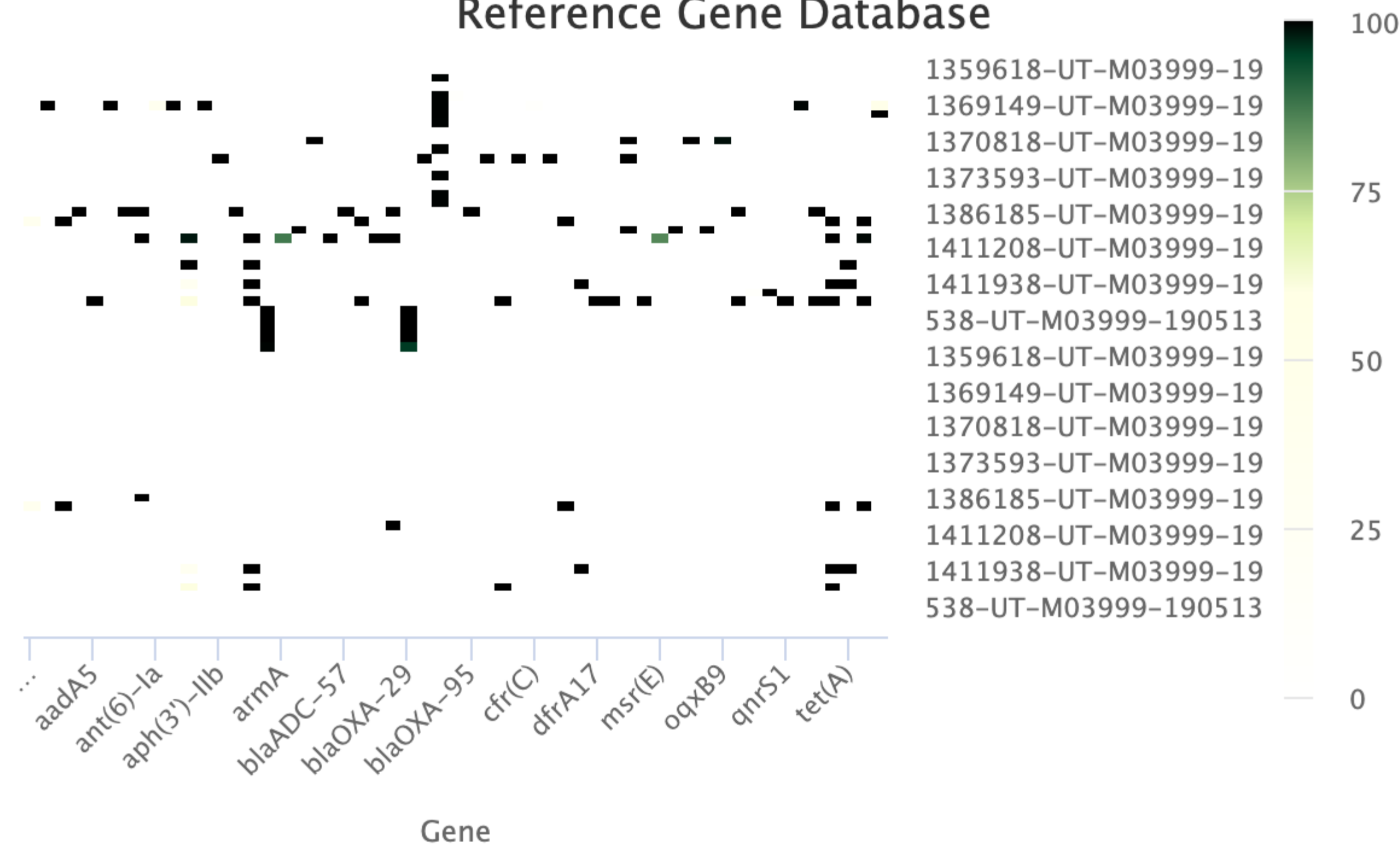


Reference Free WGS Analysis

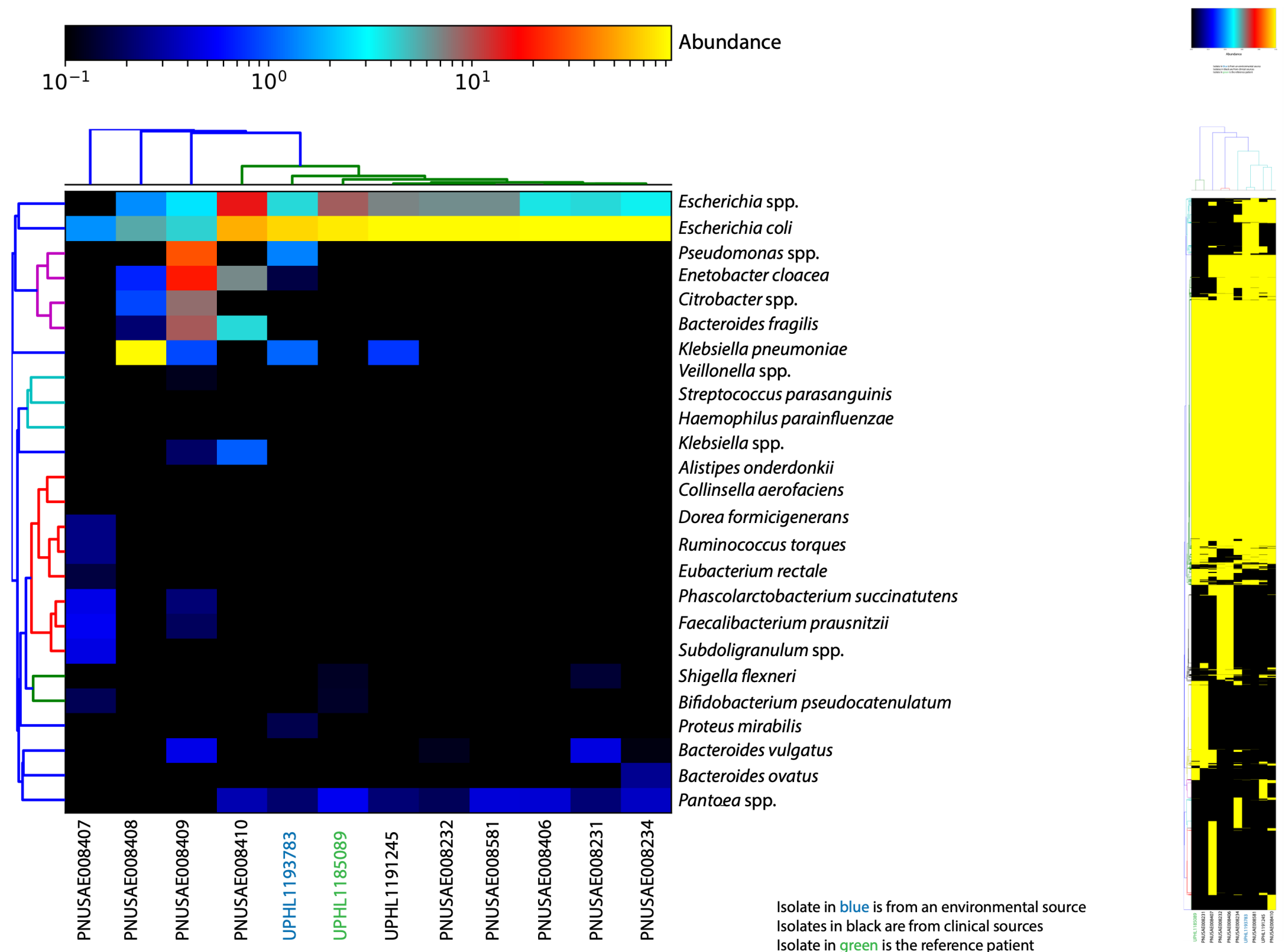


Reference Free WGS Analysis

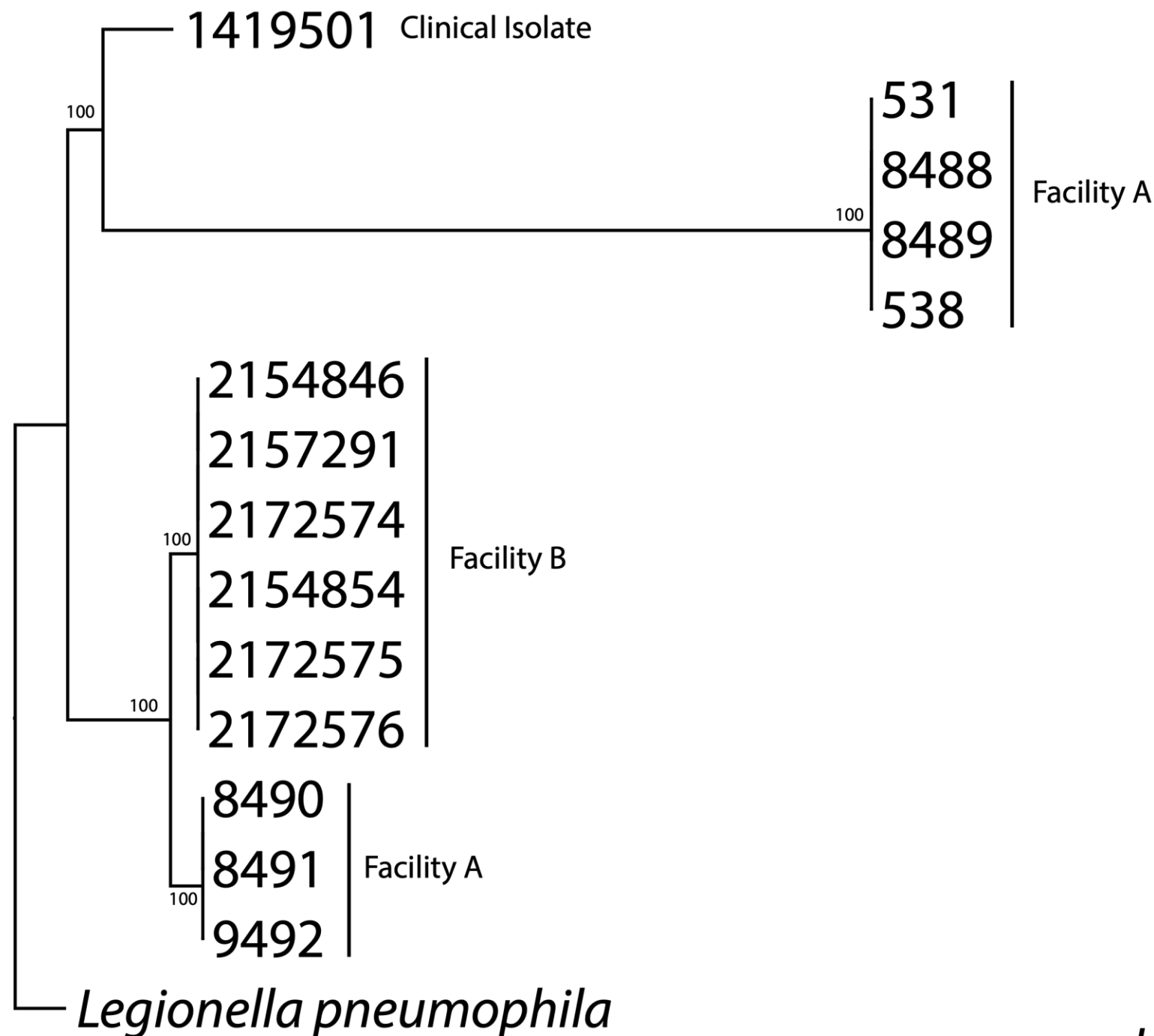
Abricate Results: NCBI Bacterial Antimicrobial Resistance Reference Gene Database



WGS & Metagenomics



Reference Free WGS Analysis



0.01 Substitutions / Site

Legionella pneumophila
Phylogenetic Tree Based on 1,356
Concatinated Protein Coding Genes

Questions/Discussion



Thank You

Utah Public Health Laboratory

Robyn Atkinson-Dunn

Andy Rohrwasser

Jenni Wagner

Anna Sangster

Erin Young



UPHL



StaPH-B
State Public Health Bioinformatics

